Methods: Primary human chondrocytes from human donors (mean ± SD 76,75 ± 3,30 years, n=3) were transfected with siRNA for Atg5 (100 nM, 72 hours), an important autophagy marker, and silencer negative control siRNA (100 nM) used as control to block the autophagy pathway. To identify the key proteins responding to defective autophagy, we performed a quantitative proteomics analysis of autophagy-deficient human chondrocytes using labeling iTRAQ (isobaric tags for relative and absolute quantitation) coupled with on-line 2D LC/MS/MS. Protein identification and quantification were performed using Protein Pilot Software v 4.0 (ABSciex). Each MS/MS spectrum was searched in the Uniprot/Swissprot database for Homo sapiens. The String database was employed to find putative protein-protein interactions. To validate the candidate targets identified by the proteomic screening, inmortalized human chondrocytes (Tc28a2), Human cartilage from healthy, aged and osteoarthritis human patients and mouse knee joints from young and old mice were employed to perform Western Blot (WB), Inmunofluorescence (IF) and Inmunohistochemistry (IHC) analysis, respectively. The candidate targets were: Atg5 and LC3 for constitutive autophagy, p62, as a defective autophagy marker, Lamin A/C as an aging marker and Filamin A, as a cytoskeleton marker.
Results: From the total of 1216 proteins found, 21 were significantly altered ($p < 0.05$) in at least two donors from a total number of three. However, only prelamin A/C, a nuclear protein implicated in premature cell senescence, was significantly upregulated in all the donors ($p < 0.05$). Furthermore, proteins involved in the cytoskeleton organization, collagen catabolism, oxidative stress, and aging were identified in deficient autophagy chondrocytes. Interestingly, the String Database Software indicates a direct interaction between Filamin A, a cytoskeleton protein ($p < 0.05$) and Prelamin A/C, an aging marker, that were downregulated and upregulated ,respectively when autophagy is blocked. To confirm this association, TC28a2 human chondrocytes were transfected with siAtg5 and silencer negative control siRNA. Then, the expression of autophagy proteins, such as Atg5 and LC3, the levels of p62, Lamin A/C and Filamin A was evaluated. The results indicated a reduction in autophagy expression, accompanied with an increase in p62 and Lamin A/C and a reduction in Filamin A expression. Importantly, in human cartilage from both aged and OA patients, autophagy markers were significantly downregulated and Lamin A/C expression was upregulated, compared to healthy cartilage. To stablish the validity of these results, articular cartilage from a spontaneous aging mice model was studied. Histology analysis of mouse knee joints from young mice (4 months old mice) and old mice (28 months old) revealed a reduction in Atg5 and LC3 expression, as well as an increase in Lamin A/C expression, suggesting that autophagy loss-of-function is directly correlated with premature senescence in articular cartilage.
Conclusions: Proteomics analysis of joint cells and tissue has revealed features of premature senescence when autophagy is disrupted in chondrocytes and cartilage. Lamin, nuclear protein contributing to structural integrity to the nucleus and matrix was identified as candidate targets for regulating cartilage function in situations of defective autophagy, including aging and OA. These results support the hypothesis that autophagy is decreased with aging, affect nuclear and matrix structural integrity, and represents a key mechanism in the development of cartilage degradation. Therefore, targeting lamin is a promising strategy to find novel therapeutics for cartilage aging and OA.

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EVALUATION OF THE BENEFIT OF CORTICOSTEROID INJECTION PRIOR TO EXERCISE THERAPY IN PATIENTS WITH KNEE OSTEOARTHRITIS: A RANDOMIZED TRIAL

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Purpose: Combined non-pharmacological and pharmacological treatment is recommended as optimal management of knee osteoarthritis (OA). However, the two treatment approaches have mostly been investigated separately. We aimed to assess the efficacy of combined intra-articular corticosteroid injection and exercise compared to placebo injection and exercise in patients with knee OA.
Methods: This randomized, double blind, placebo-controlled trial running over 26 weeks was designed as a superiority study comparing the efficacy of a single intra-articular corticosteroid injection (1 mL of 40 mg/mL methylprednisolone dissolved in 4 mL 10 mg/mL Lidocaine) plus exercise, with a single placebo injection (1 mL isotonic saline mixed with 4 mL 10 mg/mL Lidocaine) plus exercise. Participants with clinical and radiographic knee OA were randomly allocated (1:1) to either corticosteroid (Steroid Group) or saline (Placebo Group) injection. Two weeks after injection, all participants started a 12 week supervised exercise program with 3 weekly sessions. Outcomes were assessed at baseline, week 2 (exercise start), week 14 (end of exercise), and week 26 (follow-up). The primary outcome was the mean change in KOOS pain subscale at week 14. Analyses were done on the intention-to-treat (ITT) population (all randomized participants). Missing data were replaced using multiple imputation. A repeated measures mixed model was used to analyze the primary outcome; week, treatment, and week x treatment were included as fixed effects, adjusting for the baseline value.
Results: A total of 263 patients were screened and 100 patients were randomized to receive either Steroid (n=50) or Placebo (n=50). Of these, 93 completed the week 14 assessment and 89 completed the 26 weeks trial. There were no group differences in the proportions completing. Mean age was 63.4 (SD 9.3) years, 61% were women. The mean exercise attendance rate was 79% (SD 15); no group difference observed. The mean (SD) KOOS pain score at randomization was 53.3 (11.4) and 55.2 (16.0) in the Steroid and Placebo groups, respectively. The mean (SE) change in pain at week 14 was 13.6 (1.8) and 14.8 (1.8) in the Steroid and Placebo Groups, respectively, corresponding to a mean difference of 1.2 units (95% CI -3.8 to 6.2; P=0.64). These results were robust according to all sensitivity analyses - incl. using baseline observation carried-forward imputation and no imputation. There were no group differences at week 2, 14, and 26 in any of the 5 KOOS subscales and the 95% confidence intervals of the group differences did not exceed the suggested minimal clinical important difference of 8-10 KOOS points.
Conclusions: These results show comparable efficacy of intra-articular corticosteroid and placebo when combined with exercise for pain relief in knee OA.
Trial registration. EU clinical trials register (EudraCT): 2012-002607-18 and clinicaltrials.gov NCT01945749

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THE COMBINED EFFICACY OF MULTIMODAL NON-SURGICAL TREATMENT ON PAIN AND SENSITIZATION IN PATIENTS WITH KNEE OSTEOARTHRITIS NOT ELIGIBLE FOR A TOTAL KNEE ARTHROPLASTY – AN ANCILLARY ANALYSIS FROM A RANDOMIZED CONTROLLED TRIAL

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Purpose: To report the efficacy of a 12-week treatment program consisting of neuromuscular exercise, education, diet, insoles and pain medication (the MEDIC-treatment) compared to two leaflets with information and treatment advice (usual care) in reducing pain-related measures and sensitization in patients with knee osteoarthritis (OA) not eligible for total knee arthroplasty (TKA).
Methods: This was a pre-defined ancillary analysis of the 12-week results from a randomized controlled trial with 100 patients randomized to MEDIC-treatment or usual care (Trial registration: clinicaltrials.gov NCT01535001). The primary outcome was peak pain intensity in the previous 24h (VAS 0-100). Secondary outcomes included peripheral and central sensitization assessed at the knee, the lower leg and forearm (pressure pain thresholds from handheld algometry), pain intensity after 30 min of walking (VAS 0-100), pain location and pattern (Knee Pain Map), spreading of pain (a region-divided body chart) and the usage of pain medication (pain medication during the last week due to knee yes/no).
Results: 654 patients seen by an orthopaedic surgeon in secondary care were assessed for eligibility, 553 were excluded and one was not willing to undergo randomization (Primary reasons for exclusion: being eligible for TKA (n=192), not radiographic OA (Kellgren-Lawrence score<1; n=87), and inability to comply with study protocol (n=159)). 100 patients were randomized with 43/50 (86%) in the MEDIC group and 46/50 (92%) in the usual care group completing both baseline and the 12-week follow-up (see table 1 for baseline characteristics).
The MEDIC group had a mean improvement (95% CI) in peak pain intensity from baseline to 12 weeks that was 15.4 (2.6 to 28.2) larger (Figure 1; P = 0.019) than the usual care group. Furthermore, the